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Erythrocyte Alloimmunization and Autoimmunization in the Pediatric Population: A Multicenter, Cross-Sectional Study in Central China

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Keywords

Erythrocyte alloimmunization · Erythrocyte autoimmunization · Pediatric population

Abstract

Background: Erythrocyte alloantibodies and autoantibodies complicate transfusion. However, the prevalence of erythrocyte alloimmunization and autoimmunization has not been estimated in the Chinese pediatric population. Therefore, we investigated the prevalence of erythrocyte alloimmunization and autoimmunization in the Chinese pediatric population with the aim of developing a reasonable transfusion management policy in children from China. ***Methods:*** This study included 30,603 pediatric inpatients who were admitted to three tertiary hospitals in central China from May 2020 to October 2022. Antibody screening was carried out with a three-cell panel by column agglutination technology, and samples with positive screening were analyzed for antibody specificity with a 16-cell identification panel. Clinical details of the patients were collected to identify associations with antibody formation. ***Results:*** The alloimmunization rate was 0.55% (169/30,603), and the autoimmunization rate was 0.14% (43/30,603). Alloantibodies comprised 80.09% of the antibodies. The most frequent alloantibodies were anti-M (58.77%), anti-E (9.48%), and anti-P1 (4.27%). Autoantibodies comprised 19.91% of antibodies. Age ($p = 0.000$), sex ($p = 0.016$), geographical area ($p = 0.000$), ABO blood group ($p = 0.008$), and diagnosis ($p = 0.000$) were independent risk factors for antibody formation. The risk of antibody formation at the ages of 0–28 days and 1–3 months was zero (odds ratio = 0.000). The antibody distribution was significantly different by age ($p = 0.000$) and diagnosis ($p = 0.000$). ***Conclusion:*** Repeat pre-transfusion testing for infants less than 4 months of age can be omitted for no risk of antibody formation. MNS system antibodies, especially anti-M, are prominent in younger children, and this decreases with age. Provision of extended phenotype-matched transfusion for Rh system antigens, especially antigen E, is necessary in children to control erythrocyte alloimmunization. The presence of antibodies with high evanescence rates in the pediatric population suggests the pressing need for nationwide shared transfusion records to avoid hemolytic transfusion reactions in children.

Introduction

Erythrocyte alloimmunization and autoimmunization always complicate transfusion therapy with the development of unexpected erythrocyte antibodies, including alloantibodies and autoantibodies.

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Alloantibodies and autoantibodies can provoke hemolytic transfusion reactions. The severity of these reactions can vary from mild, with reduced efficacy of transfusion therapy, to extremely severe, causing rapid death of the transfusion recipient. Alloantibodies and autoantibodies can also result in difficulty locating compatible red blood cell (RBC) units for future transfusion and be clinically significant in future pregnancies, potentially resulting in hemolytic disease of the fetus and newborn (HDFN) [1, 2].

Erythrocyte alloimmunization and autoimmunization are associated with female sex. The risk of alloimmunization and autoimmunization increases with the number of RBC transfused, and there are considerable geographical and racial variations in alloimmunization and autoimmunization rate [3, 4]. However, most studies of erythrocyte alloimmunization and autoimmunization focused on adults or on specific disease groups, especially patients with hemoglobinopathy [5, 6]. There are limited data on erythrocyte alloimmunization and autoimmunization among the pediatric population, even in US and European populations, with the least data in East Asian populations.

Children are a special population, and they deserve more attention in the field of transfusion medicine. A better understanding of erythrocyte alloimmunization and autoimmunization in the pediatric population will help control the formation of alloantibodies and autoantibodies. This control will mitigate the harm of hemolytic transfusion reactions, transfusion delay, or HDFN in the children's future. The prevalence of erythrocyte alloimmunization and autoimmunization has not yet been established for the Chinese pediatric population. In the present study, we investigated a large cohort of pediatric inpatients from central China to determine the prevalence of erythrocyte alloimmunization and autoimmunization in the Chinese pediatric population. Our findings will hopefully help develop a reasonable transfusion management policy in children from China.

Material and Methods

Study Design

This was a multicenter, cross-sectional study that used data recorded in three participating medical facilities from central China. A total of 32,102 pediatric patients admitted to Second Xiangya Hospital in Hunan Province, Tongji Medical College Affiliated Wuhan Children's Hospital in Hubei Province, and Children's Hospital Affiliated of Zhengzhou University in Henan Province from May 2020 to October 2022 were enrolled in this study. Patients who developed erythrocyte alloantibodies or autoantibodies were included in the case group. Patients who did not develop erythrocyte alloantibodies or autoantibodies were selected as the control group. Patients' data, such as age, sex, geographical area, ABO blood group, diagnosis, transfusion history, transfusion episodes, transfused units, and antibody specificity, were collected from the electronic laboratory information system of the three hospitals. This study was approved by the Ethics Committees of Second Xiangya Hospital of Central South University.

Antibody Testing

EDTA-anticoagulated blood samples from these patients were collected by standardized venipuncture. Plasma samples were screened for the presence of unexpected erythrocyte antibodies using a three-cell panel of reagent group O RBCs (Ortho Clinical Diagnostics, USA). In the indirect anti-globulin test, we used LISS-enhanced column agglutination technology (Ortho Clinical Diagnostics, USA), which includes a polyspecific anti-human globulin (rabbit anti-IgG and monoclonal anti-C3d). When antibody screening had a positive result, antibody identification was performed using a commercial panel of 16 reagent cells (Sanquin Reagents, The Netherlands) of selected phenotypes with the same method. Advanced investigations, such as enzyme treatment of RBCs, adsorption, and elution, were used if indicated.

Statistical Analysis

Statistical analysis was carried out using SPSS 22.0 Statistical Software (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA). Continuous variables are shown as the mean and standard deviation, and the *t* test was applied for comparison between the two groups. Categorical variables are shown as the percentage, and the χ^2 test was used for comparison among multiple groups. Adjusted standardized residuals were then calculated to further determine how significant each of every group was to the χ^2 value, and an absolute value of adjusted standardized residuals ≥3 was considered to be statistically significant. A logistic regression analysis was applied to determine risk factors for erythrocyte alloimmunization and autoimmunization in pediatric inpatients using estimated odds ratios (ORs) with 95% confidence intervals. p values <0.05 were considered statistically significant.

Results

A total of 9,655 pediatric inpatients from Second Xiangya Hospital, 10,192 from Tongji Medical College Affiliated Wuhan Children's Hospital, and 12,255 from the Children's Hospital Affiliated of Zhengzhou University were enrolled in this study. After excluding 1,425 patients because of poorly described essential information, 30,677 patients were included with complete data. A further analytical screening process excluded 9 patients owing to duplicated detection, 45 with mother-derived antibodies, and 18 with undetermined antibodies. A total of 30,603 pediatric inpatients were ultimately included in this study (Fig. 1).

Prevalence of Erythrocyte Alloimmunization and Autoimmunization in Pediatric Inpatients

There were 169 (0.55%) cases with alloantibodies and 43 (0.14%) with autoantibodies among the 30,603 pediatric inpatients. The alloimmunized or autoimmunized patients produced 14 types of antibodies alone or in

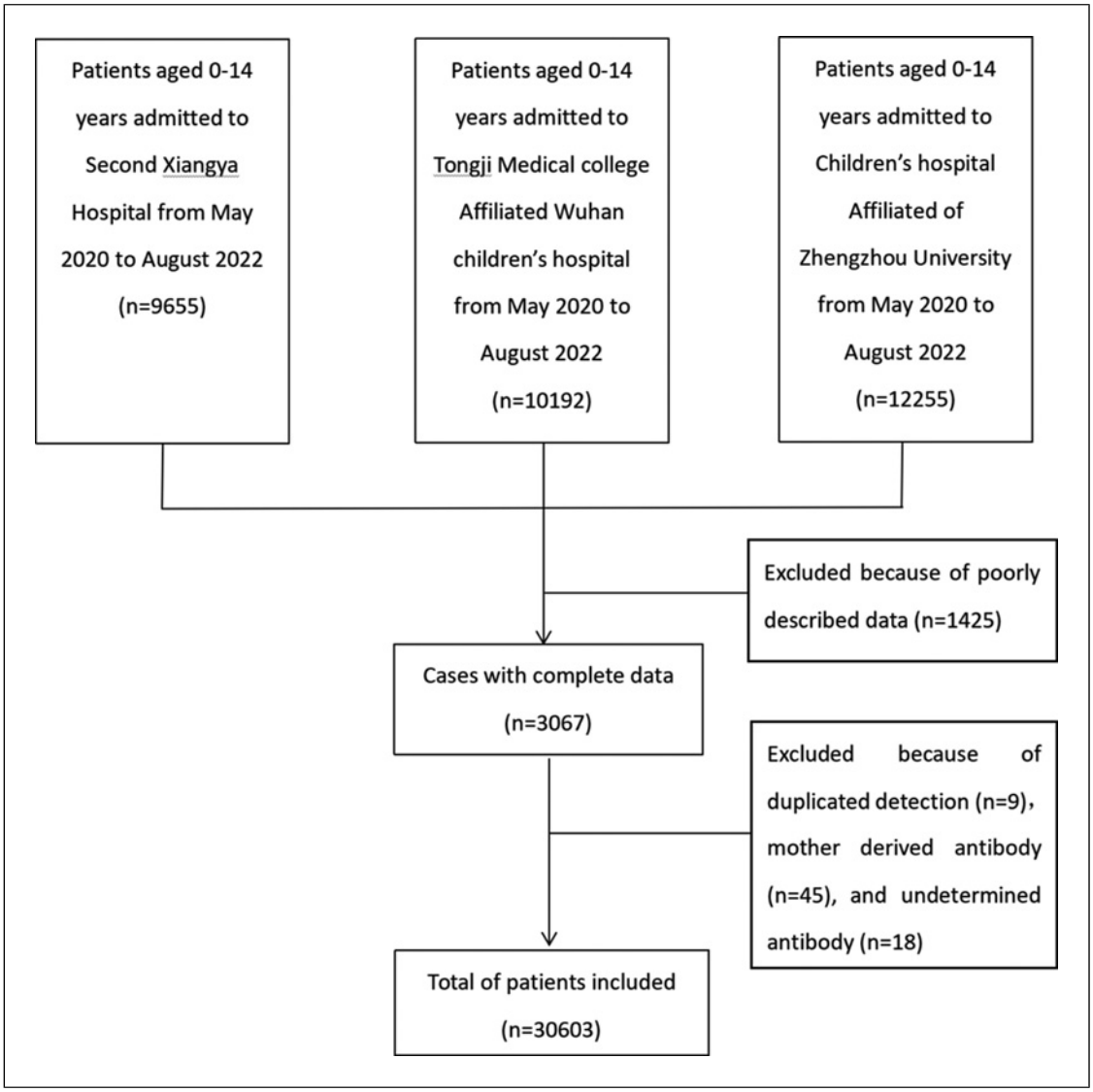

Fig. 1. Selection of the study subjects.

combination. The alloantibodies comprised 80.09% of erythrocyte antibodies. The most frequent alloantibodies were anti-M (58.77%), anti-E (9.48%), and anti-P1 (4.27%). The autoantibodies comprised 19.91% of antibodies (Table 1; Fig. 2). Combined antibodies from the same blood group system were observed in three cases (anti-Ec, anti-Ce, and anti-Lea+anti-Leb). Multiple antibodies from different blood group systems were observed in 5 cases (anti-E+anti-JKa, anti-M+anti-Lea, anti-Lea+anti-P1, anti-Jka+anti-Wra, and anti-M+autoantibody).

Demographic and Clinical Characteristics of Pediatric Inpatients

Table 2 shows the demographic and clinical characteristics of the pediatric inpatients. There were no significant differences in sex ($p = 0.273$), transfusion history ($p = 0.106$), transfusion units ($p = 0.545$), or transfusion episodes ($p = 0.821$) between the case group and control group. However, age ($p = 0.000$), geographic area ($p = 0.000$), ABO blood type ($p = 0.000$), and diagnosis ($p = 0.003$) were significantly different between the case group and control group.

Risk Factors for Erythrocyte Alloimmunization and Autoimmunization in Pediatric Inpatients

We included the abovementioned variables that showed a significance difference between the case and control groups ($p < 0.05$) in the logistic regression model and conducted a logistic regression analysis. The abovementioned variables that showed no significant difference ($p > 0.05$) were also included in the model for adjustment in case these variables play a role in antibody formation.

Table 1. Prevalence of erythrocyte alloimmunization and autoimmunization in pediatric inpatients

Blood group system	Antibody specificities	*n* (%)
Rh	Anti-E	20 (9.48)
	Anti-Ec	1 (0.47)
	Anti-C	1 (0.47)
	Anti-Ce	1 (0.47)
MNS	Anti-M	124 (58.77)
	Anti-N	2 (0.95)
P	Anti-P1	9 (4.27)
Kidd	Anti-Jk^a	1 (0.47)
	Anti-Jk^b	1 (0.47)
Lewis	Anti-Le^a	2 (0.95)
	Anti-Le^a+anti-Le^b	1 (0.47)
Diego	Anti-Di^a	1 (0.47)
Multiple antibodies	Anti-E+anti-JK^a	1 (0.47)
	Anti-M+anti-Le^a	1 (0.47)
	Anti-Le^a+anti-P1	1 (0.47)
	Anti-Jk^a+anti-Wr^a	1 (0.47)
	Anti-M+autoantibody	1 (0.47)
Autoantibody		42 (19.91)
Total		211 (100)

The logistic regression analysis showed that age (p = 0.000), sex (p = 0.016), geographical area (p = 0.000), ABO blood group (p = 0.008), and diagnosis (p = 0.000) were independent risk factors for erythrocyte alloimmunization or autoimmunization. However, the transfusion history (p = 0.273), transfusion episodes (p = 0.885), and transfusion units (p = 0.782) were not independent risk factors for erythrocyte alloimmunization or autoimmunization (Table 3).

After adjustment for the transfusion history, transfusion episodes, and transfusion units, the risk of erythrocyte alloimmunization or autoimmunization at 0–28 days and 1–3 months of age was zero (OR = 0.000). This risk increased at 4–12 months of age (OR = 1.779) and reached a peak at 1–4 years of age (OR = 2.785), and then decreased at 5–9 years of age (OR = 1.881) and further decreased at 10–14 years of age (OR = 1.000). The risk of erythrocyte alloimmunization or autoimmunization was higher in female patients (OR = 1.000) than in male patients (OR = 0.701), higher in Henan Province (OR = 1.000) than in Hubei (OR = 0.773) and Hunan (OR = 0.407) Provinces, and higher in B (OR = 0.926) and AB (OR = 1.000) blood groups than in A (OR = 0.692) and O (OR = 0.514) blood groups. This risk was also higher in immune diseases (OR = 3.790), followed by cardiopulmonary diseases (OR = 1.330), anemia (OR = 1.200), hematological diseases (OR = 0.650), and surgery/trauma (OR = 0.522), but this risk was lowest in malignancy (OR = 0.357) (Table 3).

Erythrocyte Antibody Distribution by Diagnosis, Age, Sex, and ABO Blood Group in Pediatric Inpatients

The erythrocyte antibody distribution by the diagnosis in pediatric inpatients showed that antibody specificity was associated with the diagnosis (p < 0.001). Adjusted standardized residuals showed that MNS blood group system antibodies were more likely to develop in the diagnosis of surgery/trauma (adjusted residual: >3) but less likely to develop in the diagnosis of immune diseases (adjusted residual: <−3). Rh was less likely to develop in the diagnosis of surgery/trauma (adjusted residual: <−3). Autoantibodies were more likely to develop in the diagnosis of immune diseases (adjusted residual: >3) but less likely to develop in the diagnosis of surgery/trauma (adjusted residual: <−3). Kidd blood group antibodies were more likely to develop in the diagnosis of hematological diseases (adjusted residual >3) (Table 4).

The erythrocyte antibody distribution by age in pediatric inpatients showed that antibody specificity was associated with age (p < 0.001). Adjusted standardized residuals showed that the incidence of MNS blood group system antibodies tended to rise at the age of 4–12 months (adjusted residual: <−3) and reach a peak at the age of 1–4 years (adjusted residual: >3). This incidence then declined at the age of 5–9 years (adjusted residual<−3) and further declined at the age of 10–14 years (adjusted residual: <−3). Rh blood group system antibodies were more likely to develop at the age of 10–14 years (adjusted residual: >3). Autoantibodies were less likely to form at the age of 1–4 years (adjusted residual: <−3) (Table 5).

The erythrocyte antibody distribution by sex in pediatric inpatients showed that antibody specificity was not associated with sex (p = 0.176). Similarly, the erythrocyte antibody distribution by ABO blood group in pediatric inpatients showed that antibody specificity was not associated with the ABO blood group (p = 0.104) (Tables 6, 7).

Discussion

Erythrocyte alloimmunization and autoimmunization are challenging to the transfusion process because they are associated with transfusion delays, shortened in vivo survival of donor blood and even hemolytic transfusion reactions, and HDFN [7]. While the pediatric population is a special population and more attention should be paid during the transfusion process, data of the prevalence of erythrocyte alloimmunization

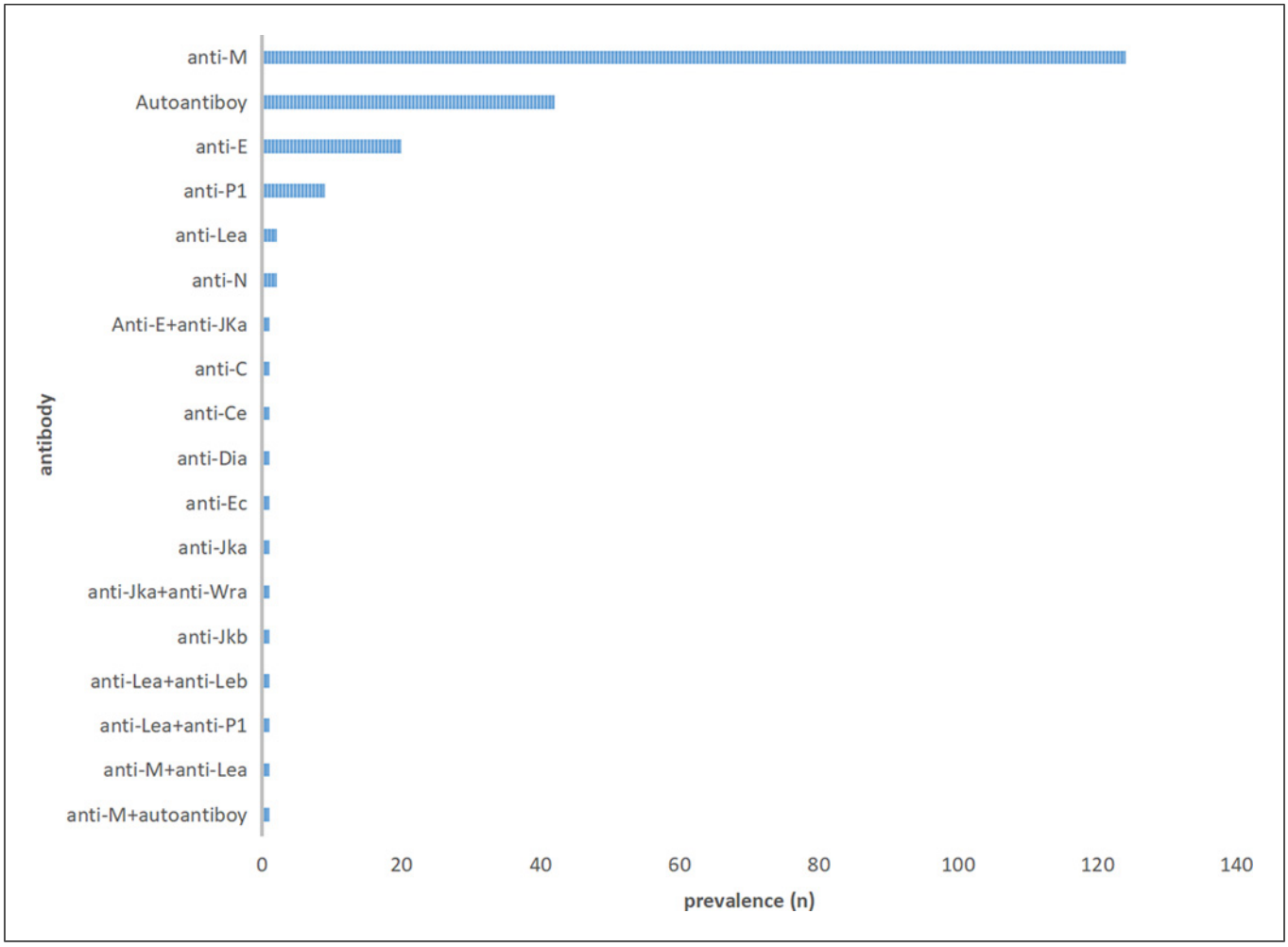

Fig. 2. Prevalence of erythrocyte alloimmunization and autoimmunization in pediatric inpatients.

and autoimmunization in this population are limited in China. We conducted a multicenter, cross-sectional study with a large sample size of 30,603 pediatric inpatients aged 0–14 years with a diverse diagnosis with or without a transfusion history in three hospitals from central China. To the best of our knowledge, this is the first multicenter study to examine the prevalence of erythrocyte alloimmunization and autoimmunization in the pediatric population in China.

In this study, the alloimmunization rate was 0.55% (169/30,603) and the autoimmunization rate was 0.14% (43/30,603). Alloantibodies comprised 80.09% of erythrocyte antibodies, and the most frequent alloantibodies were anti-M (58.77%), anti-E (9.48%), and anti-P1 (4.27%). Autoantibodies comprised 19.91% of erythrocyte antibodies (Table 1; Fig. 2). The prevalence data of erythrocyte alloimmunization and autoimmunization in literature from all over the world always generated from transfused adults, especially adults with thalassemia and sickle cell disease, and the incidence of erythrocyte alloimmunization and autoimmunization has been reported to range from 0.12% to 18%. The incidence appears to be lower in pediatric patients than in adult patients (Table 8). Tamai et al. [8] conducted a nationwide, retrospective, multicenter cohort survey in RBC recipients aged <20 years in Japan between 2001 and 2015. They found that 69 of 11,350 (0.61%) patients developed at least one clinically significant alloantibody. They found a slightly higher incidence of alloimmunization than that in our study, which can be explained by their study group of transfused patients instead of all patients. Additionally, naturally occurring, cold-reactive, and nonspecific antibodies were excluded from Tamai et al.'s study [8], which could explain why anti-M and anti-P1 were not the most frequent antibodies in their study. Poornima et al. [9] conducted a descriptive, cross-sectional study of a multitransfused pediatric population aged <18 years over 2 years in India. Alloantibodies were obtained in 4 (6.35%), and autoantibodies were obtained in 1 (1.59%) of the 63 patients. The authors found a much higher incidence of erythrocyte alloimmunization and autoimmunization than that in our study, which may have been due to exclusion of

Table 2. Demographic and clinical characteristics of pediatric inpatient

Item		Alloimmunized or autoimmunized, *n* (%)	Not alloimmunized or autoimmunized, *n* (%)	χ^2 or *t*	*p* value
Age, years	0–28 d	0 (0.00)	4,989 (100.00)	87.654	0.000
	1–3 m	0 (0.00)	1,446 (100.00)		
	4–12 m	14 (0.83)	1,678 (99.17)		
	1–4 y	120 (1.17)	10,130 (98.83)		
	5–9 y	58 (0.79)	7,241 (99.21)		
	10–14 y	19 (0.39)	4,910 (99.61)		
Gender	Male	130 (0.64)	20,163 (99.36)	2.096	0.148
	Female	81 (0.79)	10,231 (99.21)		
Geographic area	Hunan	46 (0.48)	9,552 (99.52)	28.629	0.000
	Hubei	43 (0.49)	8,768 (99.51)		
	Henan	122 (1.00)	12,074 (99.00)		
ABO blood group	A	61 (0.63)	9,557 (99.37)	15.864	0.000
	B	76 (0.90)	8,361 (99.10)		
	O	47 (0.48)	9,771 (99.52)		
	AB	27 (0.99)	2,705 (99L.01)		
Diagnosis	Anemia	28 (0.47)	5,984 (99.53)	19.713	0.003
	Surgery/trauma	103 (0.69)	14,829 (99.31)		
	Hematological diseases	15 (0.81)	1,844 (99.19)		
	Malignancy	7 (0.52)	1,340 (99.48)		
	Immune diseases	7 (2.73)	249 (97.27)		
	Cardiopulmonary diseases	20 (0.65)	3,036 (99.35)		
	Others	31 (0.99)	3,112 (99.01)		
Transfusion history	Without	177 (0.66)	26,617 (99.34)	2.613	0.106
	With	34 (0.89)	3,777 (99.11)		
Transfusion episodes (times, mean±SD)		0.92±3.68	0.79±3.28	0.606	0.545
Transfusion units (IU, mean±SD)		1.10±4.05	1.02±5.25	0.226	0.821

the population aged 0–5 months and a small sample size of only 63 patients. However, they also found that alloantibodies against Rh antigens were the main antibodies, which is consistent with our results. Türkmen et al. [10] conducted a retrospective cohort study of patients who received transfusion at a single university medical center in Germany. A total of 1,641 neonates and children up to the age of 3 years who received their first RBC transfusion between 1994 and 2013 were included, and they found that only 2 patients developed anti-M and anti-E post-transfusion at the ages of 181 and 611 days. The low alloimmunization rate found in their study was reasonable in consideration of the undeveloped immune system in their study subjects with a very young age. El Kababi et al. [11] conducted a retrospective study from 2009 to 2018 on 160 patients with thalassemia who were transfused regularly in Morocco. Their alloimmunization rate was 8.75%. The 17 alloantibodies that were identified were mainly anti-K, anti-E, anti-D, Kp^a, and anti-C. The reason for their high alloimmunization rate was probably because their study subjects were patients with thalassemia who received frequent transfusions. Except for Rh antibodies, they found that anti-K, which was peculiar in their population, was the most frequent antibody.

Avoiding unnecessary blood tests is essential for infants and neonates to prevent repeated puncture and iatrogenic blood loss [8]. The British Committee for Standards in Haematology Guidelines on transfusion for fetuses, neonates, and children recommends not repeating antibody screening. Additionally, blood compatible with the ABO type of the neonate can be used without further testing until 4 months of age following a negative direct anti-globulin test and no detectable maternal anti-A or anti-B. The USA-based AABB standards for blood banks and transfusion services have similar advice for testing of infants younger than 4 months of age, except for no requirement of direct anti-globulin testing. In the multiple logistic regression analysis in our study, we observed that the risk of alloimmunization or autoimmunization at 0–28 days and 1–3 months of age was zero (OR = 0.000) (Table 3). An immature immune system and some form of an acquired immune tolerance to allogeneic RBC antigens may be responsible for

Table 3. Risk factors for erythrocyte alloimmunization and autoimmunization in pediatric inpatients

Item	Group	Alloimmunized or autoimmunized, *n* (%)	Not alloimmunized or autoimmunized, *n* (%)	OR (95% CI)	*p* value
Age, years	0–28 d	0 (0.00)	4,989 (100.00)	0.000 (0.000–0.000)	0.000
	1–3 m	0 (0.00)	1,446 (100.00)	0.000 (0.000–0.000)	
	4–12 m	14 (0.83)	1,678 (99.17)	1.779 (0.880–3.598)	
	1–4 y	120 (1.17)	10,130 (98.83)	2.785 (1.692–4.584)	
	5–9 y	58 (0.79)	7,241 (99.21)	1.881 (1.110–3.187)	
	10–14 y	19 (0.39)	4,910 (99.61)	1.000	
Gender	Male	130 (0.64)	20,163 (99.36)	0.700 (0.521–0.940)	0.018
	Female	81 (0.79)	10,231 (99.21)	1.000	
Geographic area	Hunan	46 (0.48)	9,552 (99.52)	0.407 (0.273–0.608)	0.000
	Hubei	43 (0.49)	8,768 (99.51)	0.733 (0.503–1.068)	
	Henan	122 (1.00)	12,074 (99.00)	1.000	
ABO blood type	A	61 (0.63)	9,557 (99.37)	0.692 (0.438–1.094)	0.006
	B	76 (0.90)	8,361 (99.10)	0.926 (0.595–1.442)	
	O	47 (0.48)	9,771 (99.52)	0.514 (0.319–0.829)	
	AB	27 (0.99)	2,705 (99L.01)	1.000	
Diagnosis	Anemia	28 (0.47)	5,984 (99.53)	1.200 (0.714–2.017)	0.000
	Surgery/trauma	103 (0.69)	14,829 (99.31)	0.522 (0.343–0.793)	
	Hematological diseases	15 (0.81)	1,844 (99.19)	0.650 (0.349–1.211)	
	Malignancy	7 (0.52)	1,340 (99.48)	0.357 (0.156–0.818)	
	Immune diseases	7 (2.73)	249 (97.27)	3.790 (1.629–8.821)	
	Cardiopulmonary diseases	20 (0.65)	3,036 (99.35)	1.330 (0.734–2.410)	
	Others	31 (0.99)	3,112 (99.01)	1.000	

CI, confidence interval.

the reduced antibody risk in these patients. It concludes that the guidelines developed in Europe and the USA are applicable in Chinese patients. Using blood samples obtained from mothers instead of neonates for pre-transfusion testing is reasonable, and repeated pre-transfusion testing for infants younger than 4 months of age can be safely omitted, provided maternal blood group antibodies are not detectable [12].

Some studies have suggested that female sex is a risk factor for the production of alloantibodies and autoantibodies. In the multiple logistic regression analysis in our study, we also found that the risk of erythrocyte alloimmunization and autoimmunization was higher in female patients (OR = 1.000) than in male patients (OR = 0.701) (Table 3). However, the antibody distribution by sex showed no association between antibody specificity and sex ($p > 0.05$) (Table 6). Additionally, a higher risk of erythrocyte alloimmunization and autoimmunization was observed in B and AB blood groups (OR = 0.926 and 1.000) than in A (OR = 0.692) and O (OR = 0.514) blood groups in the multiple logistic regression analysis (Table 3). However, the antibody distribution by ABO blood group showed no association between antibody specificity and the ABO blood group ($p > 0.05$) (Table 7). Antigen B is likely to be related to antibody formation, although it is not related to which blood group system antibody is formed. Further investigation of this issue is required.

The multiple logistic regression analysis in our study showed that the risk of erythrocyte alloimmunization and autoimmunization was higher in immune diseases (OR = 3.790), followed by cardiopulmonary diseases (OR = 1.330), anemia (OR = 1.200), hematological diseases (OR = 0.650), and surgery/trauma (OR = 0.522), but malignancy had a low risk (OR = 0.357) (Table 3). Previous studies have shown that clinical circumstances surrounding RBC transfusion could affect the likelihood of the recipient becoming alloimmunized [1]. With dysfunction of the immune system, the risk of erythrocyte alloimmunization and autoimmunization in immune diseases is always higher than that in other diseases. Furthermore, patients with an inflammatory state may be more likely to become alloimmunized than patients who are healthy. The majority of cardiopulmonary diseases and anemia diagnosed in our study were caused by inflammation from infections. This explains the high risk of antibody formation in cardiopulmonary diseases in our study. However, patients with immunosuppressive treatment were less likely to

Table 4. Erythrocyte antibody distribution by diagnosis in pediatric inpatients

Antibody blood group system	Anemia	Surgery/ trauma	Hematological diseases	Malignancy	Immune diseases	Cardio pulmonary diseases	Others	χ^2	*p* value
Rh, *n* (%)	6 (26.09)	4 (17.39)	4 (17.39)	1 (4.35)	1 (4.35)	1 (4.35)	6 (26.09)	90.036	0.000
Adjusted residual	1.9	−3.2	2	0.3	0.3	−0.9	1.6		
MNS, *n* (%)	11 (8.73)	81 (64.29)	6 (4.76)	5 (3.97)	0 (0.00)	9 (7.14)	14 (11.11)		
Adjusted residual	−2.4	5.5	−1.6	0.6	−3.3	−1.4	−1.8		
P, *n* (%)	0 (0.00)	7 (77.78)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (22.22)		
Adjusted residual	−1.2	1.8	−0.8	−0.6	−0.6	−1	0.7		
Kidd, *n* (%)	0 (0.00)	0 (0.00)	2 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)		
Adjusted residual	−0.6	−1.4	5.1	−0.3	−0.3	−0.5	−0.6		
Lewis, *n* (%)	1 (33.33)	2 (66.67)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)		
Adjusted residual	1	0.6	−0.5	−0.3	−0.3	−0.6	−0.7		
Diego, *n* (%)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (100.00)		
Adjusted residual	−0.4	−1	−0.3	−0.2	−0.2	−0.3	2.4		
Multiple antibodies, *n* (%)	1 (20.00)	1 (20.00)	1 (20.00)	0 (0.00)	0 (0.00)	1 (20.00)	1 (20.00)		
Adjusted residual	0.4	−1.3	1.1	−0.4	−0.4	0.8	0.3		
Autoantibody, *n* (%)	9 (21.43)	8 (19.05)	2 (4.76)	1 (2.38)	6 (14.29)	9 (21.43)	7 (16.67)		
Adjusted residual	1.7	−4.3	−0.7	−0.4	4.4	3	0.4		

develop alloantibodies or autoantibodies. This may explain a low risk of alloantibody and autoantibody formation in the diagnosis of malignancy. Patients with hematological diseases undergo chemotherapy as well. Therefore, the risk of erythrocyte alloimmunization and autoimmunization was relatively low in these patients, even though they were under frequent stimulation from blood transfusion.

With regard to the antibody distribution by diagnosis, MNS blood group system antibodies were more likely to develop in surgery/trauma (adjusted residual: >3) but less likely to develop in immune diseases (adjusted residual: <−3) (Table 4). Most of the patients diagnosed with surgery/trauma were only admitted to hospital for surgery without any complicated medical problems, which suggested that most MNS blood group antibodies in our study were naturally occurring antibodies. In Tamai et al.'s study [13], anti-M was most frequently detected in a cohort of children aged 1 to <5 years. They concluded that naturally occurring anti-M was characteristic of a younger age, predominantly between 1 and 3 years. In our study, the antibody distribution by age in pediatric inpatients showed that MNS blood group system antibodies (most were anti-M) tended to rise from the age of 0–28 days (adjusted residual: <−3), reached a peak at the age of 1–4 years (adjusted residual: >3), and then declined to at the age of 10–14 years (adjusted residual: <−3) (Table 5). These results are in accordance with Tamai et al.'s study [13]. Naturally occurring anti-M is produced almost exclusively in young children after infections of *Hemophilus influenzae*, *Proteus mirabilis*, *Staphylococcus aureus*, *Neisseria meningitis*, etc. Anti-M is postulated to form and disappear in the course of bacterial infections among young children without any transfusion, possibly owing to cross-reactivity between some bacterial epitopes and M antigen [14, 15]. Moreover, an increasing number of HDFN cases caused by alloanti-M have been reported in the past 2 decades, especially in the Japanese and Chinese populations [16]. In non-ABO HDFN cases reported in the Chinese population, MNS antibodies were second common compared with Rh antibodies [16]. Similar to anti-K, anti-M is able to suppress erythropoiesis through inhibiting the growth of M-positive erythroid precursor cells, and hydrops fetalis or stillbirth occurs [17, 18]. Fortunately, however, our study showed that MNS blood group system antibodies mainly naturally occurred as the IgM subtype and declined at the age of 10–14 years (adjusted residual: <−3). It is unnecessary to worry that these alloantibodies might lead to HDFN in future pregnancy in girls. Previous reports also showed that erythrocyte alloimmunization and autoimmunization were associated with the transfusion history. While the incidence of erythrocyte antibodies per unit transfused is <1%, erythrocyte antibodies have a cumulative incidence of up to 35% in patients who receive frequent transfusions. We did not find that erythrocyte alloimmunization and autoimmunization were related to the transfusion history, transfusion episodes, or transfusion units ($p > 0.05$) (Table 2). This lack of finding is probably due to the large proportion of naturally occurring anti-M in our study (Table 1; Fig. 2).

Table 5. Erythrocyte antibody distribution by age in pediatric inpatients

Antibody blood group system	4–12 m	1–4 y	5–9 y	10–14 y	χ^2	*p* value
Rh, *n* (%)	0 (0.00)	11 (47.83)	5 (21.74)	7 (30.43)	51.987	0.000
Adjusted residual	−1.4	−0.9	−0.7	3.8		
MNS, *n* (%)	7 (5.56)	87 (69.05)	28 (22.22)	4 (3.17)		
Adjusted residual	−0.8	4.3	−2.1	−3.6		
P, *n* (%)	0 (0.00)	4 (44.44)	4 (44.44)	1 (11.11)		
Adjusted residual	−0.8	−0.8	1.2	0.2		
Kidd, *n* (%)	0 (0.00)	1 (50.00)	1 (50.00)	0 (0.00)		
Adjusted residual	−0.4	−0.2	0.7	−0.4		
Lewis, *n* (%)	0 (0.00)	0 (0.00)	2 (66.67)	1 (33.33)		
Adjusted residual	−0.5	−2	1.5	1.5		
Diego, *n* (%)	0 (0.00)	1 (100)	0 (0.00)	0 (0.00)		
Adjusted residual	−0.3	0.9	−0.6	−0.3		
Multiple antibodies, *n* (%)	0 (0.00)	5 (100)	0 (0.00)	0 (0.00)		
Adjusted residual	−0.6	2	−1.4	−0.7		
Autoantibody, *n* (%)	7 (16.67)	11 (26.19)	18 (42.86)	6 (14.29)		
Adjusted residual	2.9	−4.5	2.5	1.3		

Table 6. Erythrocyte antibody distribution by gender in pediatric inpatients

Antibody blood group system	Male	Female	χ^2	*p* value
Rh, *n* (%)	11 (47.83)	12 (52.17)	9.270	0.176
Adjusted residual	−1.4	1.4		
MNS, *n* (%)	80 (63.49)	46 (36.51)		
Adjusted residual	0.7	−0.7		
P, *n* (%)	8 (88.89)	1 (11.11)		
Adjusted residual	1.7	−1.7		
Kidd, *n* (%)	0 (0.00)	2 (100)		
Adjusted residual	−1.8	1.8		
Lewis, *n* (%)	2 (66.67)	1 (33.33)		
Adjusted residual	0.2	−0.2		
Diego, *n* (%)	0 (0.00)	1 (100)		
Adjusted residual	−1.3	1.3		
Multiple antibodies, *n* (%)	3 (60.00)	2 (40.00)		
Adjusted residual	−0.1	0.1		
Autoantibody, *n* (%)	26 (61.90)	16 (38.10)		
Adjusted residual	0	0		

In the antibody distribution by diagnosis, Rh antibodies were less likely to develop in surgery/trauma (adjusted residual: <−3) (Table 4). As mentioned above, most of the patients diagnosed with surgery/trauma were only admitted to hospital for surgery without any complicated medical problems. This finding suggested that Rh blood group antibodies in our study were immune antibodies instead of naturally occurring antibodies. In the antibody distribution by age, Rh blood group system antibodies were more likely to develop at the age of 10–14 years (adjusted residual: >3) (Table 5). However, the multiple regression analysis showed that the risk of erythrocyte alloimmunization and autoimmunization increased at the age of 4–12 months (OR = 1.779) and reached a peak at the age of 1–4 years (OR = 2.785). This risk then decreased at the age of 5–9 years (OR = 1.881) and further decreased at the age of 10–14 years (OR = 1.000). Surprisingly, MNS blood group antibodies showed the same trend in variation by age (Table 3). The trend in variation of immune Rh blood group system antibodies might have already been covered by MNS blood group antibodies because of the large amount of

Table 7. Erythrocyte antibody distribution by ABO blood group in pediatric inpatients

Antibody blood group system	A	B	O	AB	χ^2	*p* value
Rh, *n* (%) Adjusted residual	8 (34.78) 0.7	5 (21.74) −1.5	6 (26.09) 0.5	4 (17.39) 0.7	25.035	0.104
MNS, *n* (%) Adjusted residual	29 (23.02) −2.3	54 (42.86) 2.5	29 (23.02) 0.3	14 (11.11) −0.9		
P, *n* (%) Adjusted residual	2 (22.22) −0.5	5 (55.56) 1.2	0 (0.00) −1.6	2 (22.22) 0.9		
Kidd, *n* (%) Adjusted residual	0 (0.00) −0.9	1 (50.00) 0.4	0 (0.00) −0.8	1 (50.00) 1.6		
Lewis, *n* (%) Adjusted residual	1 (33.33) 0.2	1 (33.33) −0.1	0 (0.00) −0.9	1 (33.33) 1.1		
Diego, *n* (%) Adjusted residual	1 (100.00) 1.6	0 (0.00) −0.8	0 (0.00) −0.5	0 (0.00) −0.4		
Multiple antibodies, *n* (%) Adjusted residual	2 (40.00) 0.6	2 (40.00) 0.2	1 (20.00) −0.1	0 (0.00) −0.9		
Autoantibody, *n* (%) Adjusted residual	18 (42.86) 2.2	8 (19.05) −2.6	11 (26.19) 0.7	5 (11.90) −0.2		

Table 8. Comparison of studies in pediatric population from literature review

Item	This research	Tamai et al.'s research [8]	Poornima et al.'s research [9]	Türkmen et al.'s research [10]	El Kababi et al.'s research [11]
Year	2020–2022	2001–2015	2016–2018	1994–2013	2009–2018
Geographic area	China (multicenter)	Japan (multicenter)	India	Germany	Morocco
Subjects	Pediatric population (transfused or not transfused)	Transfused pediatric population	Transfused pediatric population	Transfused pediatric population	Transfused thalassemic pediatric patients
Age group	0–14 y	0–14 y	6 m–18 y	0–3 y	1–25 y
Sample size	30,668	11,350	63	1,641	160
Allo- or autoantibody rate	0.55% for alloantibodies and 0.14% for autoantibodies	0.61% for alloantibodies	6.35% for alloantibodies and 1.59% for autoantibodies	0.12% for alloantibodies	8.75% for alloantibodies
Main antibody	Anti-M, anti-E, and anti-P1	Anti-E Anti-E + c Anti-C + e	1 each of anti-E, anti-c, anti-C^w, and anti-D + anti-C	1 each of anti-M and anti-E	Anti-K, anti-E, anti-D, Kp^a, anti-C
Remarks		Naturally occurring, cold-reactive, and nonspecific antibodies were excluded			

naturally occurring anti-M formation in our study. Under this situation, an older age does not indicate a lower risk of immune Rh blood group antibody formation. In contrast, as the immune system gradually matures, the risk of immune Rh blood group antibody formation increases with age. The majority of Rh blood group system antibodies were anti-E in our study (Table 1; Fig. 2). Therefore, provision of extended phenotype-matched transfusion for Rh blood group antigens, especially for anti-E, in children older than 4 months (particularly 10–14 years) was necessary to control the erythrocyte alloimmunization.

In the antibody distribution by diagnosis, autoantibodies were more likely to develop in the diagnosis of immune diseases (adjusted residual: >3) but less likely to develop in

the diagnosis of surgery/trauma (adjusted residual: <−3) (Table 4). This finding can be explained by the relatively healthy body in surgery/trauma, but there is dysfunction of the immune system in immune diseases. However, in the antibody distribution by age, autoantibodies were less likely to develop at the age of 1–4 years (adjusted residual: <−3) (Table 5). Further investigation is required to determine if there is immune tolerance at the age of 1–4 years.

Importantly, evanescence is a phenomenon that affects many different alloantibodies according to antigenic specificity. Some of the highest evanescence rates have been reported for alloantibodies to antigens in the Kidd (Jk^a and Jk^b), Lutheran (Lu^a), and Kell blood groups (Js^a) [1]. Antibody levels diminish over time, and negative antibody screening is commonly found in patients with a history of antibodies. Unfortunately, antibodies of the Kidd blood group, which are known as antibodies with the highest evanescence rates, were found in our study in the pediatric population. These antibodies were more likely to develop in hematological diseases (adjusted residual: >3) in which long-term transfusion is always required (Table 4). In China, there is no nationwide antibody registry. Most hospitals do not have access to a shared registry of antibodies previously detected at other hospitals. Therefore, a patient might be transfused at one hospital and have erythrocyte alloantibodies or autoantibodies detected, but he/she subsequently receives care at another hospital. If the antibody detected at the first hospital evanesces or falls below the level of detection by the time the patient is admitted to the second hospital, then an apparently compatible RBC transfusion could result in a rapid anamnestic erythrocyte immunization response and lead to hemolytic transfusion reactions. Therefore, a national registry of erythrocyte alloimmunization and autoimmunization needs to be developed, especially for the pediatric population, to help improve transfusion safety and outcomes. With a shared registry, patients will be provided with a record of the antibody identified, which can be accessed across the health system to enable selection of suitable blood in the future, even if antibodies decrease to undetectable levels.

This study has some limitations. First, because undetermined antibodies were excluded in our study, the majority of which were probably multiple antibodies that were difficult to identify, the incidence of multiple antibodies may have been higher. Second, considering the kinetics of development and evanescence of some alloantibodies, the incidence of alloimmunization was likely to be underestimated. Third, results from multiple centers may have been affected by diverse identification techniques at each institution. A third-party identification agency may help obtain more reliable results in further studies.

In summary, repeated pre-transfusion testing for infants aged younger than 4 months can be omitted with no risk of antibody formation. MNS system antibodies, especially anti-M, are characteristic of young children, and they decrease at older ages. Provision of extended phenotype-matched transfusion for Rh system antigens, especially antigen E, is necessary in children to control erythrocyte alloimmunization. The presence of antibodies with high evanescence rates in the pediatric population highlights the need to promote nationwide shared transfusion records to avoid hemolytic transfusion reactions in children.

Statement of Ethics

This study was approved and has been granted an exemption from requiring written informed consent by the Ethics Committees of Second Xiangya Hospital of Central South University (No.20191009).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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None.

Author Contributions

Yongjun Wang, Hongbin Hu, and Ding Zhao designed and organized the study. Zhengfeng Li and Wei Li collected the study data. Yuanqing Yang analyzed the data and wrote the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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